in "dipstick" diagnoses. These antibodies can be used early in the infection, well before more conventional tests. Using dipstick technology, where plastic sticks are coated with monoclonal antibodies, it will eventually be possible to dip the sticks in body fluids and actually fish the disease agent out. After immersion, the dipstick is rinsed in a series of short baths; a color change is produced if the agent is present.

Eventually, this type of monoclonal-antibody-based dipstick technology will provide additional information to producers. It will provide information about the level of contamination of feed with mycotoxins or pesticides. It also will be of benefit in defining whether potentially harmful drug residues, antibiotics, or carcinogens have contaminated milk, meat, or poultry products. In the future essentially anywhere a substance, organism, or compound needs to be detected, measured, or monitored, monoclonal antibodies will play a role.

Research

In the animal world, especially in research, monoclonal antibodies are being used in purifying compounds, tracing milk synthesis during lactation and in understanding the origin and development of various disease-producing agents, including the role of certain proteins and enzymes in cancer. Rapid and accurate monoclonal-antibody-based pregnancy tests for cows and horses are on the way. Even the possibility of presexing embryos fertilized in test tubes looms in the future.

Indeed the potential use of monoclonal antibodies to assure unequaled animal health, to promote agriculture in general, and to provide more wholesome products for the consumer is only limited by our imagination and the speed with which these reagents may be created.

Genetic Engineering Can Help Control Disease

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Infectious diseases are still the main cause of illness and death in domestic livestock. The estimated annual loss incurred from infectious disease in cattle and swine exceeds 1 billion dollars and for all livestock species exceeds 2 billion dollars.

The widescale use of therapeutic drugs and biologics, such as antibiotics and vaccines, also has added to the cost of raising livestock. Yet they have reduced the losses only marginally because of the changes in agricultural production methods which often promote the occurrence of infectious disease. The concentration of animals in feed lots, dry lot dairies, integrated swine operations, and broiler production units are examples of husbandry that increase the spread of infectious agents as compared with less confined types of animal raising.

Methods of Control

Three major methods are used to reduce losses from infectious disease. One is to eliminate the infectious agent from the environment so that animals will not be exposed. This requires destroying any animal infected with the organism, whether it be the host animal or an intermediate host, such as an insect that carries the organism. This has been effective in controlling infectious agents that are not highly transmissible and that may not survive long periods outside the host. Examples are the eradication in the United States of hog cholera in swine and smallpox in humans.

The low-level feeding of antimicrobial drugs is another way to control infectious agents, especially in newborn animals. The development of resistant strains of many micro-organisms to a number of antimicrobials has caused concern although this method is still widely used.

A third method of control is to immunize animals with a form of the organism that will induce an immune response. This requires the use of vaccines containing an immunogen that induces persisting protection against the invading organism.

Actually, all three methods may be used in some form, but the third method, immunization, offers the greatest promise. It is simple, inexpensive, requires few administrations, and, most importantly, prevents infection and, therefore, minimizes damage that often accompanies infection. Greater safety and effectiveness in newer biological products will enhance their use.

Immune System

To understand how vaccines protect, it is important to understand how the immune system defends an animal against an infectious agent. (Editor's note: To conserve space, the author's explanation of the body's immune system was eliminated, and the reader's attention is directed to an explanation of that system in Gary A. Splitter's article.)

Vaccines in Current Use

Instead of allowing infections to occur naturally, it has long been the practice to expose animals to infectious agents artificially so that they will develop antibodies and be protected against the common disease-causing organisms. Thus, many vaccines have been developed and used in animals to control the more serious infectious diseases. The vaccines in use now are made from either attenuated living organisms or inactivated organisms.

The attenuated living organisms have reduced virulence, are required only in small amounts, and, in general, induce long-lasting immunity. They elicit a controlled subclinical infection and, in general, are very effective. Occasionally, however, they produce side effects that may be as severe as the natural infection.

Inactivated vaccines are safe in that they do not contain any infectious material, but they are weak in terms of stimulating an immune response. They usually require multiple injections over several weeks to induce an immune response comparable to that induced by living organisms. They also may cause undesirable side effects evident both at the site of inoculation and sometimes as a general side reaction as the animal responds adversely to the many antigenic components in the vaccine.

In other words, the use of whole organisms in either the living or inactivated form may cause adverse reactions. These reactions are due to certain components of the whole organism, that is, proteins, lipids or carbohydrates that may not be necessary for immunization.

Synthetic Vaccines

Biosynthetic Process. The objective of vaccine development over the years has been to identify the important antigens responsible for protection and to produce them in the purest form. But only recently has recombinant DNA technology, (rDNA, genetic engineering) become available to help produce defined antigens, or antigenic determinants, on a large scale and in a cost-effective manner. The isolation of these antigenic determinants on the surface of infectious agents represents the first step in trying to produce a more specific antigen. Since these determinants occur in repeating subunits and their production is controlled by specific genes in the nucleus of the organism, these genes may be used to produce antigenic determinants.

By isolating the specific gene (DNA) that encodes for the surface antigenic determinant, and by using a plasmid (a piece of DNA that occurs naturally in bacteria and yeast) to insert this gene as a bacteria, yeast, or mammalian cell, the gene recombines with the cell's own genes to produce the antigenic determinant along with other cellular products. The antigenic determinant may be isolated and used as an immunogen. This immunogen will be recognized by the immune system as being foreign and will stimulate the production of antibodies or a cellular response that will protect the animal or prepare the animal's immune system for future infection with the infectious agent.

Antigenic determinants can be produced by growing the cells on a large scale and collecting and purifying the antigen as it is expressed. The antigen may have improved characteristics compared to the antigen derived from the whole organism. These characteristics are purity, safety, and stability. Also, the risk of having the vaccine contaminated with infectious material used in production of the whole organism is reduced. All of these characteristics help in developing improved vaccines.

Chemical Synthesis. Another method of producing antigenic determinants is chemical synthesis. Most antigenic determinants are proteins composed of chains of amino acids. Individual amino acids may be linked together in a linear form to mimic antigenic determinants. So, if the amino acid sequence of the native antigenic determinant is known, it can be made synthetically.

One way to determine the amino acid sequence of an antigenic site is to isolate the gene that encodes for it. The gene is composed of DNA that contains the genetic code in its nucleotide sequence. This nucleotide sequence can be determined, and it will translate into an amino acid sequence (the bases, adenine, thymine, guanine and cytosine code in triplet combination for each amino acid). Thus, an amino acid sequence for a surface protein may be derived from the nucleotide sequence of its gene. Only a small part of this surface protein may be required to produce an immunogen.

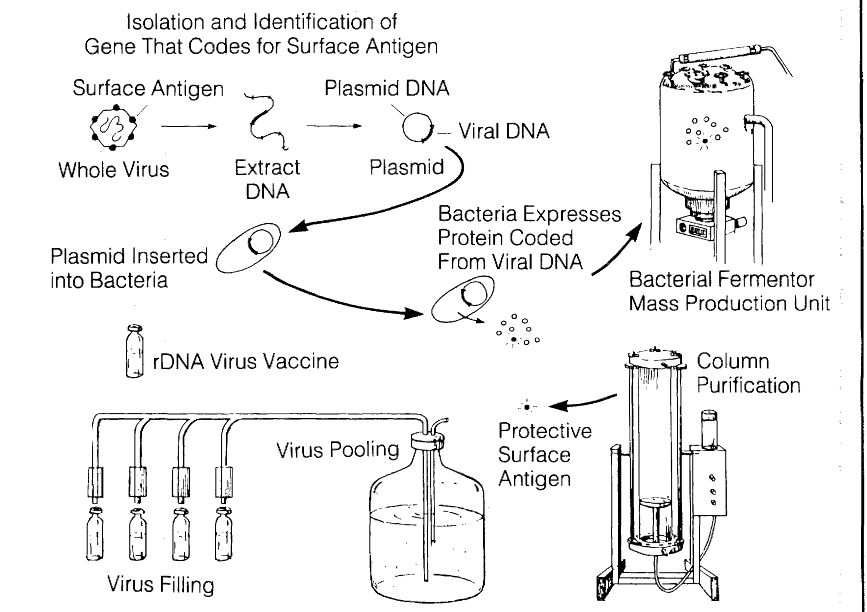
The peptide can be made by sequentially adding amino acids. Forty or 50 amino acids may be joined together in a linear sequence forming a peptide by using an amino acid synthesizer controlled by a computer program. The peptide is removed from the resin and may be coupled to a carrier protein or polymerized to increase its size. These forms of the antigenic determinant have been found to be active in inducing humoral and cellular immune responses.

The advantage of chemically synthesized peptides over biosynthesized peptides is that the chemical process is more precise and reduces the variability found in a biological process. This precision leads to further improvement in purity. There also is no chance that an infectious agent or foreign nucleic acid will find its way into a chemically synthesized product.

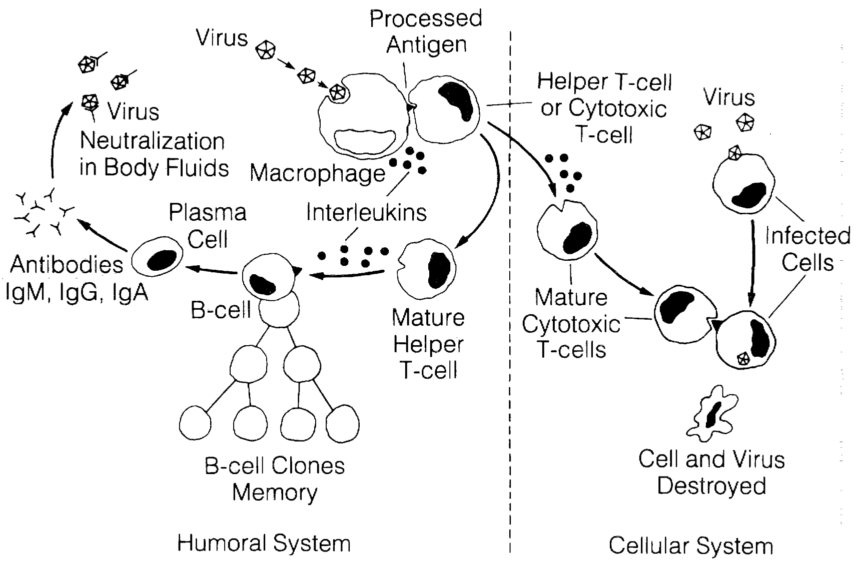
Other Approaches to Improve Vaccines

The use of live attenuated organisms as vaccines to produce a controlled infection has been the major method

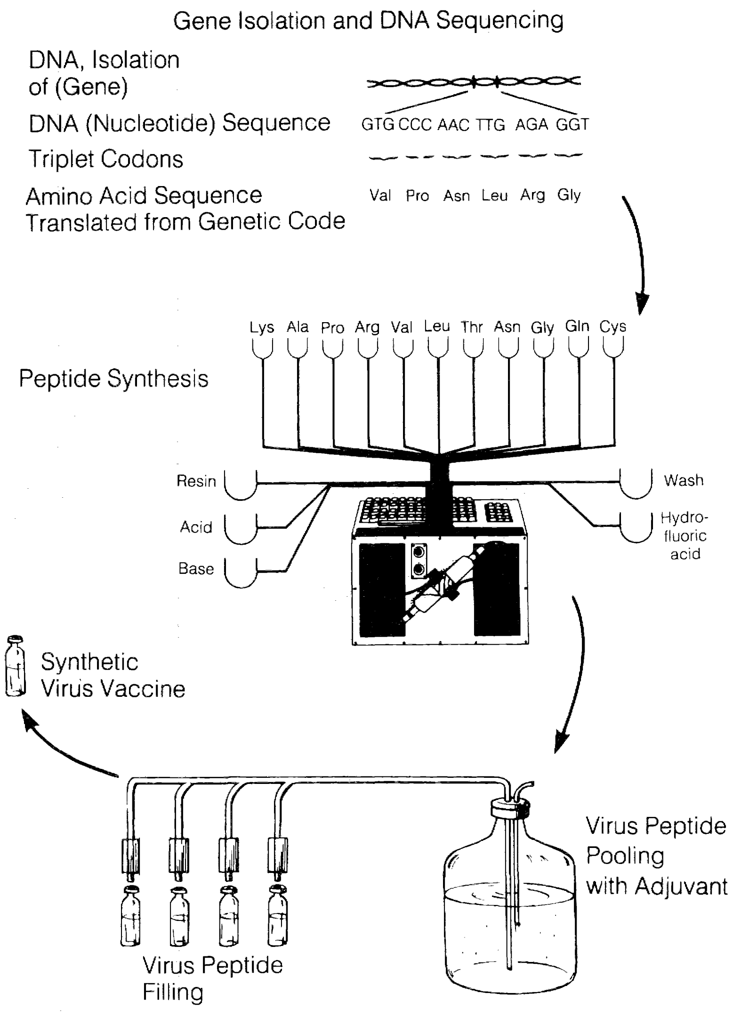
rDNA Virus Production



Immune Response to Viral Infection



Synthetic Virus Production



of protecting animals and humans. This type of vaccine usually yields a long-lasting immune response with one inoculation. Although there is some risk that the organism used in this type of vaccine may revert to a more virulent form or that the individual animal may respond adversely to the organism, the benefit usually outweighs the risk incurred.

Recombinant technology offers a method of improving this type of vaccine by recombining genes for immunodominant antigens from other infectious agents with the genes normally found in the organism used in the vaccine. Vaccinia (cowpox virus) has been the most widely studied virus used for this purpose because of its large set of genes and wide host range. It will infect many animal species by replicating, causing antibodies to be produced against both the vaccinia virus and also against other proteins encoded by the inserted DNA.

A number of bacterial organisms also have been used for this purpose. These include *Salmonella typhi* and *Vibrio cholera*. Using genetic engineering techniques, the genes causing the virulence of these organisms may be deleted while still allowing the organism to propagate in the host animal. Genes that encode for a surface antigenic determinant may be inserted into the bacterial genome (set of chromosomes, with genes), thereby allowing the organism to express an immunodominant antigen. This type of bacterium has the advantage that its cellular material often attracts leukocytes, allowing more rapid processing of the antigens bringing an improved immune response.

The advantage of attenuated living organisms in vaccines is that they reproduce—allowing a small number of organisms to be used. These may mimic the natural organism, producing a large amount of antigen that results in a longer lasting immunity.

Use of Vaccines

Although vaccines are effective in controlling many animal diseases, many debilitating, life-threatening diseases are still endemic. Present vaccines are not always safe, are not stable (require refrigeration), and have not been developed for many infectious diseases. The new technology called genetic engineering will help solve many of these problems and make more vaccines available that are safer, more stable and more effective. Their wide-scale use as multivalent products will further reduce infectious diseases.

Immunopharmaceuticals

Relatively few pharmaceutical products are used today to regulate the immune system of animals. These products are being used as aids in vaccines to stimulate the immune response to prevent disease or to treat disease conditions such as in immunodeficiency or in chronic infection. Crude bacterial extracts from Mycobacteria, Corynebacteria, Pseudomonas and Salmonella organisms have been used. They have proved to have an immuno-stimulating effect, although their mode of action is not entirely clear. Separated fractions of these bacterial products have been shown to be more active and much safer to administer than the crude products.

Recent developments in the field of immunology have furthered our understanding of how to regulate the immune system and will lead to the development of many new pharmaceutical products. Some of these are natural products from the immune system, such as thymic hormones, monokins (interleukin 1), lymphokines (interleukin 2), and interferons. These substances modulate the immune system by causing either stimulation or suppression.

There also are chemical substances that exert a similar influence such as levamasol, isoprimosine, corticosteroids, and cyclosporin A. These are available as products but are not widely used.

It is now clear that the immune system is the master control system that has great influence over most body functions. Products that affect this system will be important in the future of animal health.